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August 1976

ANALYSIS AND GC-MS CHARACTERIZATION OF TOXAPHENE IN FISH AND WATER

by

David L. Stalling
James N. Huckins

Fish-Pesticide Research Laboratory
Fish & Wildlife Service
United States Department of the Interior
Columbia, Missouri 65201

Contract No. EPA-IAG-0153 (D)

Project Officer

Leonard H. Mueller
Environmental Research Laboratory
Duluth, Minnesota 55804

U.S. ENVIRONMENTAL PROTECTION AGENCY
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SECTION I

CONCLUSIONS

1. Toxaphene residue analysis in fish using gas chromatography with electron capture detectors required multiple cleanup techniques for reliable analysis. Gel permeation, Florisil®, and silicic acid chromatography permitted efficient separation of toxaphene from coextracted lipids and PCBs. Computer difference chromatography allowed direct comparisons of a toxaphene gas chromatograph standard with environmental residues.

2. Concentrations of toxaphene residues in water ranging from 10 to 500 ng/l were quantitively extracted with a column of polyurethane foam.

3. Environmental analysis of toxaphene residues was best accomplished using chemical ionization mass spectrometry combined with gas chromatography. Specific detection of toxaphene was only feasible by using chemical ionization mass spectrometry and specific ion monitoring techniques.

4. From chemical ionization mass spectra toxaphene was found to be composed of several homologous series of chlorinated camphenes containing 5 to 10 chlorines per molecule. For each degree of chlorination there were numerous isomers derived from three empirical formulas which differ by two hydrogen atoms, i.e., $C_{10}H_{(14-N)}Cl_N$, $C_{10}H_{(16-N)}Cl_N$, and $C_{10}H_{(18-N)}Cl_N$. The first empirical formula represents replacement of 4 hydrogen atoms with 4 chlorine atoms. This reaction may also produce 4 molecules of HCL. The second and third empirical formulas are most likely produced by the addition to camphene of 2 and 4 molecules of HCL respectively. One or two additional series of compounds existed which had atypical chemical ionization mass spectra. These compounds were characterized by a base peak of mass 243 and 245. Ion fragments from these compounds represented $C_8H_7Cl_4^+$ and $C_8H_9Cl_4^+$ respectively, and may have reflected a structural rearrangement of camphene during synthesis of toxaphene.

SECTION II

RECOMMENDATIONS

1. Gel permeation and silicic acid chromatography should be utilized for the separation of lipids and PCBs from toxaphene.

2. Concentrations of toxaphene residues in water ranging from 10 to 500 ng/l should be extracted with polyurethane foam columns.

3. Environmental residues of toxaphene should be analyzed or confirmed by chemical ionization mass spectrometry.

4. Computer difference chromatography should be utilized as a direct means to detect change in isomer composition of environmental residues of toxaphene.

SECTION III

INTRODUCTION

Analysis and chemical characterization of toxaphene is challenging to pesticide analysts due to the extreme complexity of the material. Toxaphene is a mixture of compounds produced by the chlorination of camphene; characterized by having an average empirical formula of $C_{10}H_{10}Cl_8$[1] and a corresponding ^{35}Cl molecular weight of 410. Analytical techniques for toxaphene residues were reviewed by Zweig[2] who regarded gas chromatography (GC) as the most useful of the chromatographic techniques. However the utility of GC in toxaphene residue analysis is limited by the multiplicity of constituents. Over 40 components have been resolved by the use of a support-coated open tubular (SCOT) GC column[3]. Many of these peaks are due to multiple components that are not separated with a SCOT column[4]. The complex isomer composition decreases toxaphene's GC detection limit because of the multi-component nature of chromatograms. In addition, widespread contamination from ubiquitous polychlorinated biphenyls (PCBs), which are also complex multi-isomer chemicals, often interfere with toxaphene analysis.

Chronic laboratory studies of fish exposed to toxaphene require very low concentrations (10-500 ng/l)[5] and therefore sensitive residue analysis is necessary. Sample preparation schemes should not introduce interfering contaminants and cleanup techniques should remove any other interfering materials. Methods used for residue recovery must be quantitative for individual toxaphene isomers to permit detection of any alteration in isomer ratios.

The objectives of this study were: 1) to improve techniques for toxaphene quantitation in water samples, 2) to refine or develop analytical methodology for sample preparation, cleanup, and quantitation of residues and shifts in isomeric composition, 3) to characterize the composition of toxaphene using both electron impact (EI) and chemical ionization (CI) gas chromatography-mass spectrometry (GC-MS), and 4) to determine the applicability of GC-MS for the confirmation or analysis of toxaphene in environmental fish samples.

SECTION IV

EXPERIMENTAL

REAGENTS

(a) Solvents - pesticide grade, redistilled in glass.

(b) White, porous polyurethane plugs - Gaymar Identi-plugs, fits 24 to 35 mm opening, order no. L 800.

(c) Florisil^{R} - 60-100 mesh, activated at 130 C.

(d) Silicic acid - 100 mesh analytical reagent (Mallinckrodt No. 2847).

APPARATUS

(a) Silicic acid extraction column - glass, 460 mm x 85 mm id, fitted with a 5 mm bore teflon stopcock.

(b) Silicic acid chromatography column - glass, constructed with a 300 mm x 22 mm id Kimax column (order #17800) having a removable teflon stopcock. The Kimax column was joined to a 250 ml reservoir which was fitted with a 24/40 standard female ground glass joint. A 24/40 male joint was attached to an air outlet (air is filtered through charcoal) and was held in place on the column with a Thomas standard taper clamp. The system could maintain a pressure of 5 lbs.

(c) Gel permeation chromatograph - an automated system having a 22 sample capacity[6]. Separation was achieved by a column (2.5 cm x 23 cm) of BioBeads S-X2 and cyclohexane at a flow rate of 5 ml/min.

(d) Gas chromatographs - Perkin-Elmer Model 881, equipped with Tracor ^{63}Ni-electron capture detector. Operational parameters: 213 cm (7') x 2.0 mm id coiled glass column packed with 3% (w/w) OV-7 on chromosorb W-hp; nitrogen carrier gas flow rate 40 ml/min; temperatures (C) - injection port 230, detector 330, column 180. Beckman GC-5, equipped with Tracor ^{63}Ni-electron capture detector. Operational parameters: same as those above, only change was a higher column temperature of 200 C.

(e) Gas chromatograph-electron impact-mass spectrometer (GC-EI-MS). Perkin-Elmer model 270 double focusing, low resolution EI mass spectrometer coupled through Watson-Bieman molecular separator to a temperature programmed gas chromatograph. The GC-EI-MS system was interfaced to a Digital Equipment Corporation PDP-12 LDP (8K) computer.

(f) Gas chromatograph-chemical ionization-mass spectrometers (GC-CI-MS). A modified MS-9 CI-MS[7] interfaced to a PDP-8I minicomputer. Ionizing gas was isobutane and sample access was with direct probe. Sample spectra were obtained in cooperation with Dr. Henry Fales, National Heart and Lung Institute, National Institute of Health, Bethesda, Md.

(g) A Finigan quadrapole GC-CI-MS combined with system industry "System-150" PDP 8-m computer was employed to examine GC peaks of toxaphene. The gas used for ionization was methane. Toxaphene spectra from this system were obtained in cooperation with Dr. Craig Shew, Kerr Research Center, EPA, Ada, Oklahoma.

SECTION V

RECOMMENDED METHODS

SAMPLE PREPARATION

(a) Fish - Each yearling or adult sample was prepared according to the method described by Benville and Tindle[8]. A frozen fish was cut into small pieces, 1" x 1", and ground with an equal amount of dry ice in a Waring industrial blender, until a homogenous mixture was obtained. Then the fish and ice mixture were loosely sealed in a polyethylene bag and placed in a freezer (-12 C) overnight. After the dry ice sublimed, a 20 g sample of the ground fish was mixed with 80 g of anhydrous Na_2SO_4 in a beaker. Due to contaminants, all Na_2SO_4 was heated in a muffle furnace overnight at 550 C before use. To prevent hardening the sample was occasionally stirred with a glass rod until a dry mixture was obtained.

Fry or egg samples (2 g or less) were placed in 70 ml capacity porcelain evaporating dishes, each with 8 g sodium sulfate. If an individual fry was larger than 1 g it was dissected with a scalpel before proceeding. A stainless steel rod (16 cm x 0.9 cm od) for each sample was employed to crush the tissue with $NaSO_4$. Occasional stirring was necessary to prevent hardening while drying.

(b) Water - White, porous polyurethane plugs were cleaned by solvent extraction in a 2 liter stainless steel beaker with a mixture of 250 ml of acetone-petroleum ether (1:1; v/v). A smaller 1.0 liter steel beaker with several holes in the bottom was used with a piston like action on the plugs to facilitate contaminant extraction by the solvent mixture. After several minutes of washing, the contaminanted solvent mixture was discarded and the process repeated, until chromatographic analysis of the solvent mixture showed no significant GC peaks. Three clean polyurethane plugs were pushed into a 1" id clean copper column (Fig. 1). The column was constructed from a 12" piece of copper tubing with a 1" sweat union on top and a 1" to 1/2" sweat reducer on bottom. A copper plug with 8 mm hole was inserted into the reduced end of column and silver soldered in place. A Teflon buret tip (8 mm od) was inserted into the bottom of the column with the stopcock open. Then 50 ml of acetone was poured into the column and allowed to elute.

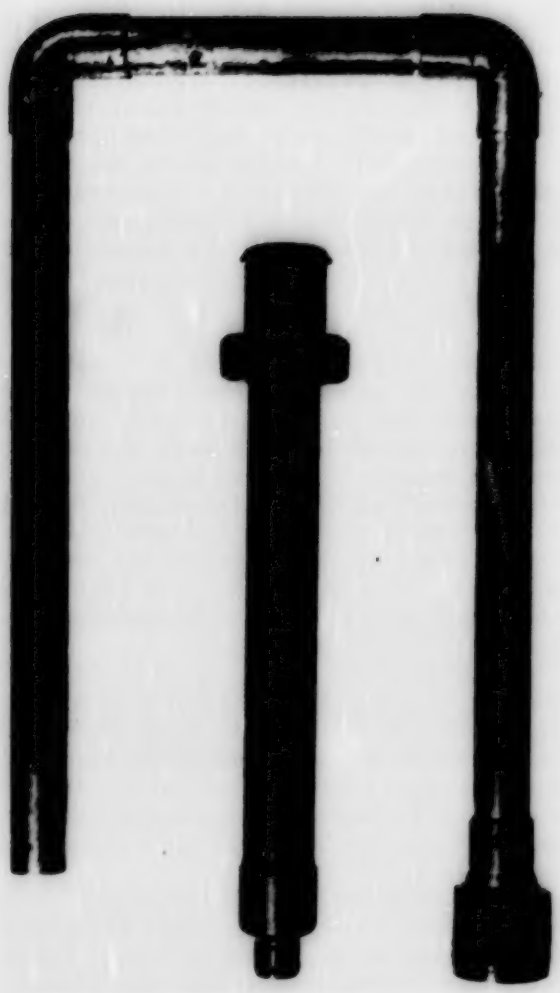

Figure 1. Water sampling apparatus consisting of a 1" id copper column (center) and siphon arm (perimeter). The left side of siphon arm from elbow down is aluminum.

This rinse was followed by 100 ml of methanol and finally, 100 ml of distilled water. Afterwards, the buret tip was removed and a siphon shown in Fig. 1 was securely coupled to the column. A small piece (2" x 2") of wire screen was inserted into the aluminum arm of the siphon to filter large particulates in the water. Aluminum pipe was used for the aquarium arm of the siphon because of the toxicity of copper to fish. The column and attached siphon were filled with water and corks were placed in both ends. Then the completed assembly was transferred to the aquaria to be sampled and the siphon was started by removing the two corks. A large wash tub, or other suitable container was calibrated to a 20 liter volume for receiving the sample eluate.

EXTRACTION

(a) Fish - After drying the yearling or adult sample - Na_2SO_4 (20 g/80 g) mixture was gently packed in a 19 mm id column of similar design to that reported by Hesselberg and Johnson[9]. Toxaphene was eluted quantitatively with 200 ml of 5% diethyl ether in petroleum ether. The elution rate should be 3 to 8 ml/min, and was controlled by packing tightness and sample texture. Then 5 ml of cyclohexane was added to the extract and it was concentrated to a 5 ml volume by evaporation on a hot plate set at 75 C in a fume hood. An additional 10 ml of cyclohexane was added and the solution re-evaporated to a 5 ml volume.

The dry fry or egg sample - Na_2SO_4 (2 g/8 g) mixture was gently packed in a 10 mm id extraction column of similar design to that used for yearling and adult fish. Toxaphene was eluted quantitatively with 100 ml of 5% diethyl ether in petroleum ether. The extract was concentrated to a 2 ml volume as described for yearling and adult samples.

(b) Water - The elution rate for the siphon-column extraction system should be 300 to 400 ml/min. However, extraction of toxaphene was quantitative up to 500 ml/min. After 20 liters of eluate had been collected the siphon was broken and the column was removed. A buret stopcock was again inserted into the bottom of the column and 75 ml of acetone was added, carefully washing down the sides of the column. Acetone removed water from the plugs which interfered with efficient extraction. The elution rate was adjusted to 5 ml/min and the eluate collected in a 250 ml separatory funnel. Then, 100 ml of petroleum ether was added to the column, and eluted into the separatory funnel at 5 ml/min, which completed extraction of the plugs.

The biphasic eluate consisted of petroleum ether on the top and acetone-H_2O on the bottom. The toxaphene in the acetone-H_2O phase was partitioned into the petroleum ether phase upon shaking of the separatory funnel. After separation of the two phases, the acetone-H_2O phase was drained into a 2nd separatory funnel. Then an additional 100 ml of petroleum ether was added to the acetone-H_2O phase and the partitioning step repeated. The two petroleum ether extracts were combined in a 400 ml beaker and dried with 20 g of anhydrous Na_2SO_4. The combined extract was then transferred to a 250 ml casserole and concentrated to 2 ml on a hot plate set at 80 C in a fume hood.

SAMPLE CLEANUP

(a) Fish - An automated gel permeation system as reported by Tindle and Stalling[6] was used for initial cleanup of yearling or adult samples. The sample was diluted to 20 ml volume in cyclohexane and 5 ml (equivalent to 5 g tissue) was loaded on the gel system. The 0 to 100 ml eluate containing lipids was discarded and toxaphene was recovered quantitatively in the 100 to 325 ml eluate. The toxaphene eluate was concentrated, as previously described, to a 5 ml volume. Changes in toxaphene isomer ratios were negligible with this method. Less than 0.5% of the original lipid remained in the collected toxaphene fraction.

Additional cleanup of yearling and adult samples was achieved by a 19 mm glass column, identical to the extraction column, with 10 g of activated (130 C) Florisil®. The sample was applied to the dry column and followed by two 5 ml washes of petroleum ether. Toxaphene was eluted with 180 ml of 5% diethyl ether in petroleum ether. The flow rate was 30 ml/min or higher and toxaphene recovery was 95-100%. The eluate was concentrated to 5 ml and silicic acid chromatography was employed if PCBs were present.

Fry or egg samples (2 g or less) contained less total lipid than yearling or adult samples and were normally purified in one step. A 10 mm glass column, used for sample extraction described earlier, was loaded with 4 g of activated Florisil followed by 0.5 g of anhydrous Na_2SO_4. The column was washed with 20 ml of petroleum ether and the sample was added when the solvent reached the top of the Na_2SO_4 layer. Toxaphene was eluted with 50 ml of 5% diethyl ether in petroleum ether. The eluate was collected in a porcelain evaporating dish (70 ml capacity) and concentrated to 5 ml on a hot plate set at 75 C. Then the eluate was transferred to a culture tube, rinsing with 5 ml of petroleum ether. The sample was

concentrated with a stream of nitrogen to an appropriate volume in a liquid bath module blok heater set at 55 C. The sample was ready for GC unless PCB contamination was present, in which case silicic acid chromatography was employed.

(b) Water - Polyurethane foam was not specific for toxaphene, and sample cleanup was usually necessary. Florisil chromatography was utilized and the same cleanup procedure as described for fry or egg samples was used with the following exceptions: Only 2 g of activated Florisil was required and toxaphene was eluted with 45 ml of 5% diethyl ether in petroleum ether.

PCB-TOXAPHENE SEPARATION

(a) Extraction and activation - 400-500 g of non-activated silicic acid was slurred, as received from manfacturer, with 1 liter of 40% acetonitrile in dichloromethane and poured into an 85 mm id x 45 cm extraction column (glass wool used as column plug) with Teflon stopcock closed. After adding an additional 2 liters of the same solvent, the flow rate was adjusted to 10 ml/min and aluminum foil was placed over top of column. Solvent percolation was completed overnight. Next, the adsorbent was transferred to a 5 liter evaporating dish and placed under a fume hood. The solvent was allowed to evaporate at ambient temperature. When dry, the crust formed on surface of silicic acid was broken with a glass stirring rod and spread to a depth of 1". The silicic acid was transferred to an oven at 160 C and heated for a minimum of 48 hrs. Care was used in making certain all solvent volatilized before heating. After activation, the adsorbent was placed in a large jar and deactivated with 2% water (98 g silicic acid + 2 ml water). Finally, the jar was sealed and tumbled on a jar mill for 1 hr. The deactivated silicic acid was allowed to equilibrate for 24 hours before use.

(b) Column preparation - 20 g of deactivated silicic acid was weighed into a 250 ml beaker. Next, the silicic acid was slurried with 70 ml of petroleum ether and poured into a 22 mm id chromatographic column with stopcock open. The column was not permitted to drain dry. Silicic acid remaining in the beaker was rinsed into the column with additional petroleum ether and the sides of the column were rinsed. Air pressure was applied (2-3 psi) and the silicic acid was allowed to settle. 2 g of Na_2SO_4 was added to the top of the silicic acid and air pressure was applied until solvent level in column was even with top of Na_2SO_4 layer.

(c) Chromatographic separation - The sample extract, previously cleaned up with gel permeation chromatography and/or Florisil, was added in not more than 5 ml of petroleum ether and pressure was applied until sample level was even with Na_2SO_4. The sides of the column were washed twice with 5 ml of petroleum ether and the solvent level was brought even with Na_2SO_4. A 275 ml capacity porcelain casserole dish was placed under the column and 250 ml of 1% benzene in petroleum ether (PCB eluate) was added. Air pressure was applied until a flow rate of 4 ml/min was achieved. The PCB eluate was collected until solvent level was 1 cm from top of Na_2SO_4 layer and elution was stopped. Another casserole was quickly placed under the column and 200 ml of 20% diethyl ether in benzene was added to column. The separation was completed by elution of the toxaphene fraction and the casserole dish containing toxaphene was transferred to an explosion proof hot plate (75 C) in a fume hood. With hood fan on, the toxaphene fraction was evaporated to a 5 ml volume and rinsed into a culture tube using 5 ml of petroleum ether. Then the sample was placed in a water bath module blok with heater set at 55 C. Using a stream of N_2, the toxaphene fraction was evaporated to a 5 ml volume.

(d) NaOH partition of pesticide fraction - If early eluting GC peaks with retention time less than p,p'-DDE were encountered in the analysis of the toxaphene fraction, an additional cleanup step was necessary. The contaminant was sometimes observed at higher GC temperatures as a broad solvent peak. To remove this contaminant, 1 ml of 1 N NaOH was added to the concentrated toxaphene fraction in a culture tube and shook thoroughly in a super (vortex) mixer for 30 seconds. The two layers were allowed to settle for 15 minutes, centrifugation was optional, and the toxaphene sample was ready for GC analysis.

TOXAPHENE QUANTITATION

(a) Total residues - Toxaphene residues were calculated by measuring three major GC peaks having retention times well separated and representative of the total residue. The GC peaks routinely used had retention times relative to p,p'-DDE of 1.48, 1.90, and 2.66. After drawing a full baseline (injection point through last sample peak), heights of the respective peaks were measured and summed. The sum of the GC peaks of three toxaphene standards covering the range of sample peak heights, were also calculated. The sum of the standard peak heights were correlated with injected quantities using a programmable Olivetti-Underwood 101 calculator to determine a linear regression curve. This standard curve was then compared with the sums of sample peak heights. Corrections for injection amounts, volume changes, and recovery were made

by the program, and the resulting residue concentration computed from the regression curve. Residues were expressed as μg/g.

(b) Isomer changes - Eight prominent peaks in a sample were selected having GC retention times representative of the total toxaphene residue. A full base line was drawn as described earlier and the heights of the eight selected peaks were measured. The heights of the same eight peaks from each of four toxaphene standards were measured. The quantity of toxaphene used for standards covered the range of sample peaks heights. Using the programmed calculator, peak heights of a sample and the standards were separately summed and individual peaks were expressed as percent of total peak height. A standard was selected whose summed peak heights was nearest that of the sample being analyzed. Corresponding peaks (% of total peak height) of the standard were then subtracted from those of the sample and results were given in $\pm$ percent deviation from a toxaphene standard.

SECTION VI

RESULTS AND DISCUSSION

The recommended methods used in this report were developed in conjunction with an EPA financed study on the residue dynamics of toxaphene in brook trout (Salvelinus fontinalis)[5]. Uptake, elimination and changes in isomer ratios of toxaphene residues in brook trout were elucidated and discussed.

WATER ANALYSIS

Various sources of PCB and phthalate ester contaminants were major obstacles in the measurement of low concentrations of toxaphene (10-500 ng/l) residues in water. These contaminants originated from solvents, adsorbents, and other unidentified sources. However, PCB and phthalate ester contamination were reduced by using redistilled solvents and solvent extraction of reagents.

The initial use of large volumes of distilled organic solvents for partition extraction of toxaphene water samples resulted in significant GC interference when concentrated to small volumes. Later we modified a column extraction method utilizing polyurethane plugs for sampling large volumes of water[10]. A rigid siphon with an attached column was used for sampling water in aquaria. The attachment of a siphon eliminated pouring large volumes of water through the polyurethane column. Recoveries of toxaphene with the siphon-polyurethane column system were as follows: 100 ng/l=100%, 50 ng/l=80%, 25 ng/l=50%. These recoveries were based on the mean of duplicate determinations for each of the three toxaphene concentrations. Organic solvent required for extraction of toxaphene utilizing polyurethane plugs was only 200 ml. Thus, the contamination from large volumes of organic solvents was decreased and time required for toxaphene analysis was greatly reduced.

FISH ANALYSIS

There were several reported techniques used for the extraction of organochlorine pesticides from fish samples for residue analysis. However, most methods were subject to serious limitations at low concentrations and often required subsequent filtering and drying steps which may introduce contaminants. The column extraction procedure described in the experimental section combined extraction, filtration and drying[9]. Recoveries of toxaphene using the extraction column ranged from 97-100% with a mean of 98.5% for three spiked samples.

The separation of lipids from pesticides often has been the most time-consuming process in pesticide analysis. Automated gel permeation chromatography of lipid-toxaphene extracts resulted in improved analytical precision, decreased manipulative sample losses, and significant saving of labor[6]. In addition all GC resolved toxaphene isomers were recovered with no apparent change in ratios. Mean recovery for three toxaphene samples was 96% and sample recoveries ranged from 95-98%. The remaining lipid or polar contaminants (less than 0.5% of original lipid content) were removed with Florisil column chromatography.

Low concentrations (0.05-0.1 ug/g) of PCBs were found in toxaphene fish sample extracts. Toxaphene's GC sensitivity was considerably less than PCBs which necessitated a PCB-toxaphene separation step. We found that only silicic acid chromatography separated all toxaphene components resolved by GC from interfering PCBs. However recent lots of silicic acid as received from the supplier were often contaminated with detectable levels of PCBs, phthalate esters, and large amounts of an unidentified early eluting (GC) contaminant. We modified the method of Stalling and Huckins[11]. The modifications eliminated many of the contaminants in silicic acid and provided reproducible separations. A large volume column (85 mm id) extraction procedure reduced PCBs and phthalates in silicic acid. The early GC eluting silicic acid contaminant was removed by partitioning the toxaphene fraction of the separation with 1 N NaOH. Additional research into the nature of this contaminant is now being conducted utilizing our GC-MS-computer system.

GAS CHROMATOGRAPHY

Research into GC solid supports and liquid phases demonstrated that OV-7 on chromosorb W-hp provided good resolution and sensitivity of toxaphene. Two GC column temperatures were used for most toxaphene analysis. A temperature of 180 C appeared optimum for isomer studies, exhibiting acceptable component resolution. However, whole body fish residues and water samples were analyzed at 200 C column temperature, a compromise of component resolution and long retention times of toxaphene samples. The minimum detection limits for toxaphene residues in fish and water utilizing the recommended methods and GC conditions described were 0.05 ug/g and 0.010 ng/l respectively.

A gas chromatograph utilized for toxaphene isomer studies was interfaced with our PDP-12 LDP computer. Multiple component chromatograms were processed by the computer as two thousand sequential data points and stored on magnetic tape using the program "CATACAL". The chromatogram data thus generated was manipulated and plotted for graphical comparisons.

DIFFERENCE CHROMATOGRAPHY

Quantitative data representing changes or differences between complex GC curves are not readily presented. Toxaphene residues were no exception due to the large number of constituents and limited GC resolution. We devised a computer based method to assist in characterization and presentation of toxaphene residue chromatograms. The technique used our PDP-12 computer and the program CATACAL to create a "difference chromatogram". A difference chromatogram was created by subtracting a standard toxaphene chromatogram from a sample chromatogram or subtraction of sample from standard chromatogram after the sample chromatogram was normalized by making its largest peak height equal to the height of the corresponding toxaphene GC peak in the standard (Fig. 2 and 3). If the two chromatograms were identical, a straight line was obtained; constituents whose relative concentration exceeds that of the standard appeared as positive peaks. Constituents which were not common to the standard were usually observed as discrete positive or negative peaks. This technique easily enabled examination of toxaphene residues for changes in isomer composition or weathering due to environmental conditions. The utility of the method was demonstrated by the preferential elimination of early eluting toxaphene components by fish 14 and 56 days after cessation of toxaphene exposure.

GC-MASS SPECTROMETRY

Computerized GC-MS helped overcome many of the difficulties in toxaphene characterization and detection. With our GC-EI-MS the computer programs "MASH" (Digital Equipment Corp. Users Manual, DEC-12-SQ-A-D) enabled rapid data acquisition, storage, and reduction of complex multi-component samples. Detection sensitivity was increased by using mass chromatograms generated by the computer from several key toxaphene fragments in sequentially acquired spectra.

A large volume sample injection system (Fig. 4) which was a modified version of SOL-VENT[12], was adapted to the GC-EI-MS allowing injection volumes of 50-100 ul without adverse effect. The system functioned to trap less volatile constituents in the liquid phase of a large diameter pre-column and vented the volatile solvents for a variable time of 15-90 seconds. After venting and closing the toggle valve, the trapped constituents were temperature programmed into the analytical column and analysis proceeded normally. A SCOT GC column was utilized when high resolution was necessary. However, a 274 cm (9') x 2 mm glass column with 3% OV-7 on chromosorb W-hp was used for routine separations.

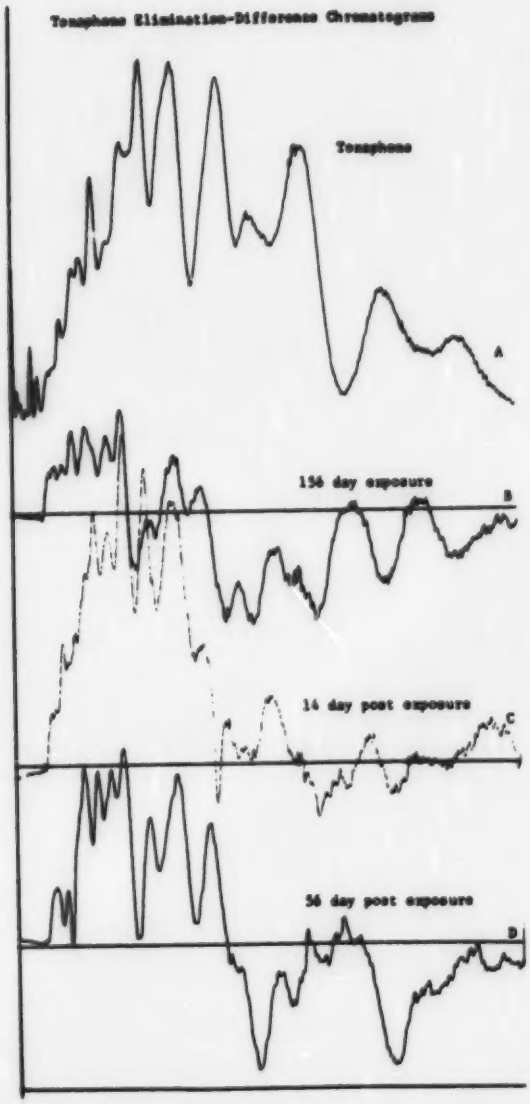

Figure 2. Difference chromatograms for toxaphene elimination from brook trout. Curve A. Toxaphene standard chromatogram. Curves B, C, and D were generated from computer subtracting of three individual GC curves representing toxaphene residues in fish after 156 day exposure, 14 day and 56 day post exposure.

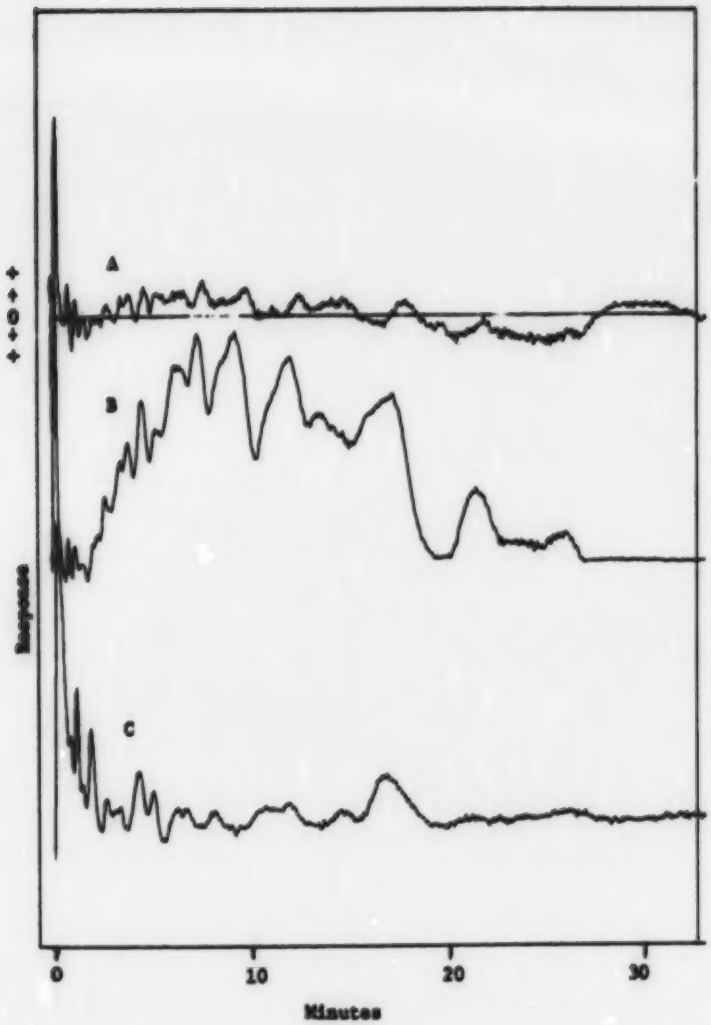

Figure 3. Curve A. Difference chromatogram obtained by subtracting toxaphene standard from the difference of curves B and C. Curve B. Toxaphene chromatogram from 20 liters of water containing 0.5 ug toxaphene/1. Curve C. Background chromatogram from 20 liters of water in control aquaria. All samples were equivalent injections into the GC.

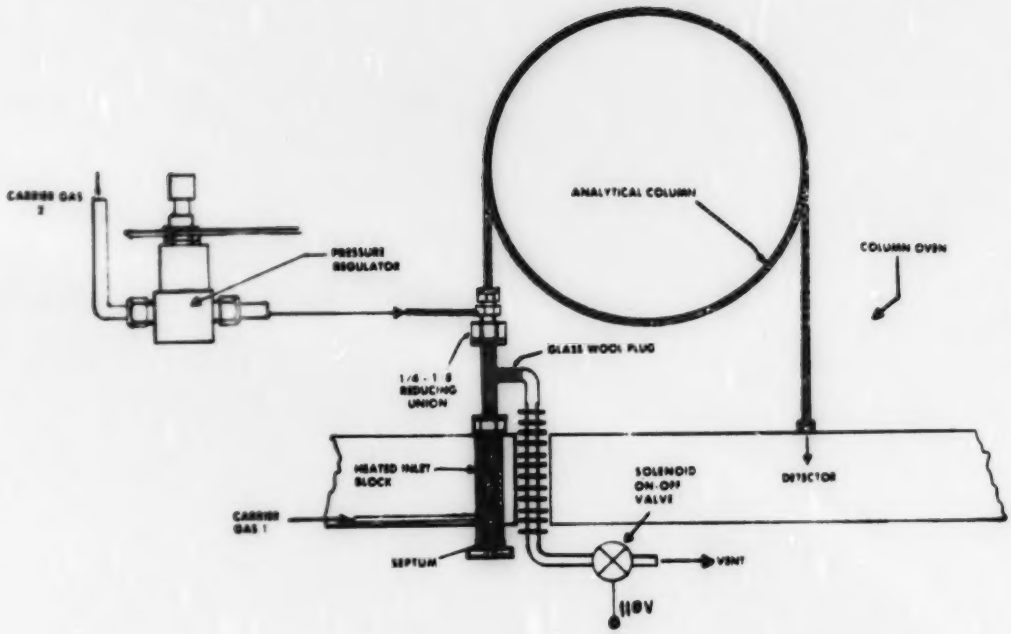

Figure 4. Large volume sample injection system for GC-MS. A pre-column with a solenoid valve is used for venting excess solvents.

Toxaphene was initially examined using our computerized GC-EI-MS to obtain spectra of GC peaks. The toxaphene peaks eluting during temperature programming were scanned over the mass range of 18 to 550 in 4 seconds at 8-second intervals for 151 scans. Data representing each spectrum was stored on magnetic tape. EI-MS spectra from several major toxaphene components were examined for characteristic ion fragments (Fig. 5). The mass chromatograms constructed from the combined ion intensities of m/e 291 + m/e 293 (Fig. 6) in 151 sequential mass scans closely resembled the peak envelope recorded from the total ion monitor. GC peaks in the mass chromatogram determined from m/e 83 (C^+HCl_2) eluted late in the chromatogram while GC peaks containing m/e 117 (C^+Cl_3) generally eluted earlier (Fig. 7). Approximately 1-2 ug of toxaphene was required for residue confirmation using EI-mass chromatograms constructed from several intense ion fragments (Fig. 8) with the aid of a large volume injection system described earlier.

Recently, mass spectrometric characterization of purified toxaphene constituents was reported by Casida et al.[4] Chemical ionization mass spectra were obtained from Casida of two purified constituents with empirical formulas of $C_{10}H_{10}Cl_8$ and $C_{10}H_{11}Cl_7$. Each CI-MS spectrum showed loss of a Cl from the molecule to form $(M-Cl)^+$ ions and $(M-Cl_2)^+$. This data permitted general correlation of empirical formula and observed CI-MS.

Examination of toxaphene by CI-MS with direct probe access resulted in spectra characterized by multiple ion clusters. These clusters represented isomeric series of $C_{10}H_5Cl_9$ with variable numbers of hydrogen for each degree of chlorine substitution (Fig. 9). The total ion curve represented the volatilization of toxaphene from the direct probe (Fig. 9). The volatilization curve based on masses in the 400-500 range was similar to the total ion current (TIC) with only slight fractionation of toxaphene during volatilization (Fig. 9). Using the intensities of the mass fragments in CI spectra, the concentration of the various isomeric series were estimated (Table 1). Two series of nonachloro toxaphene constituents ($C_{10}H_7Cl_9$ and $C_{10}H_9Cl_9$) were apparent from the direct probe examination.

After determining the applicability of CI-MS to the characterization of toxaphene, GC-CI-MS was employed to examine GC peaks. A Finnigan quadropole GC-CI-MS with a PDP 8-m computer system was used. The computer software generated reconstructed gas chromatograms (RGC) which presented specified ion-fragment intensity in each sequentially acquired MS scan as a normalized curve. This format differed from that of the MASH GC-MS system in that the latter computer programs present the gas chromatogram as a histogram. The GC-CI-MS spectra of toxaphene were several orders of magnitude simpler than corresponding GC-EI-MS spectra.

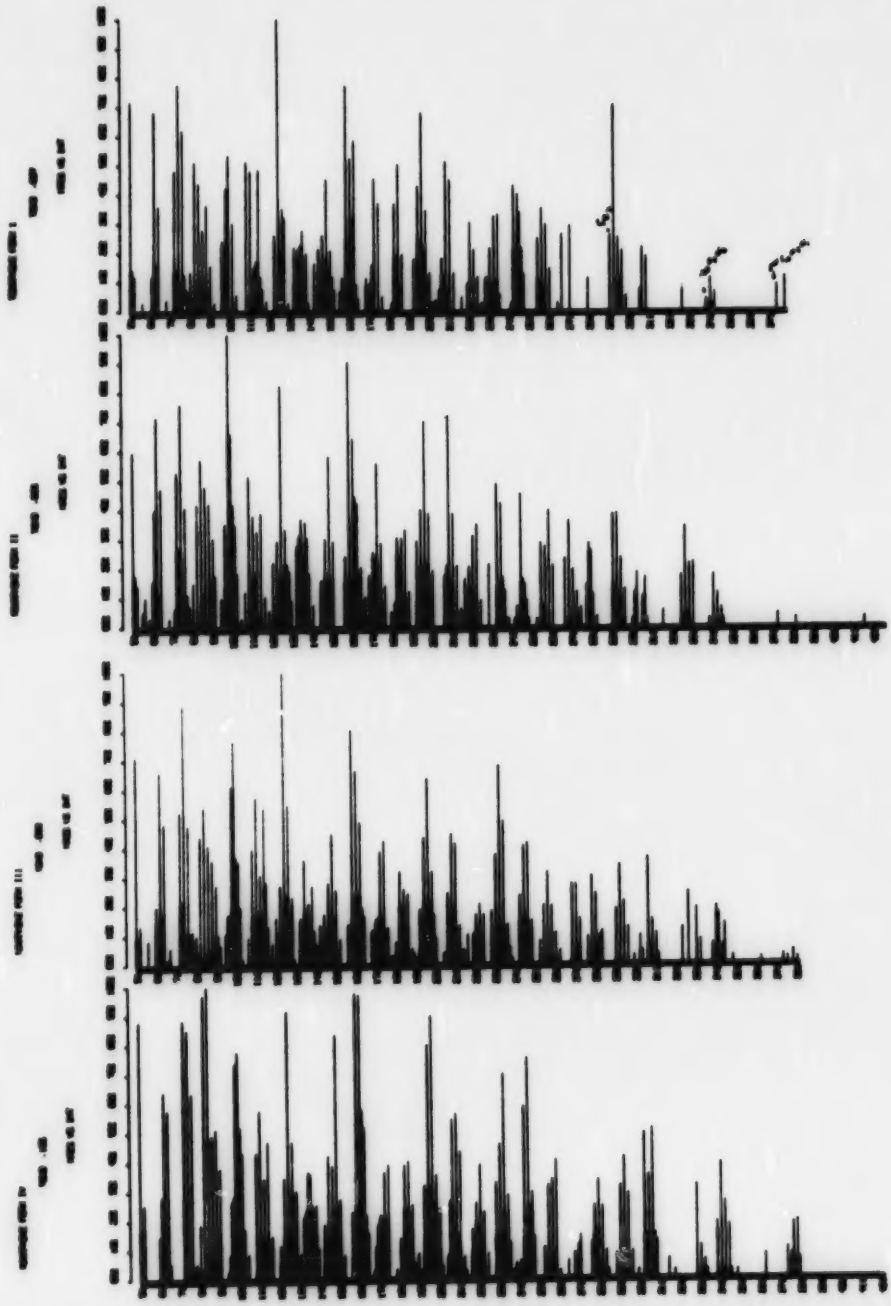

Figure 5. Characteristic EI-MS spectra of four major toxaphene constituents.

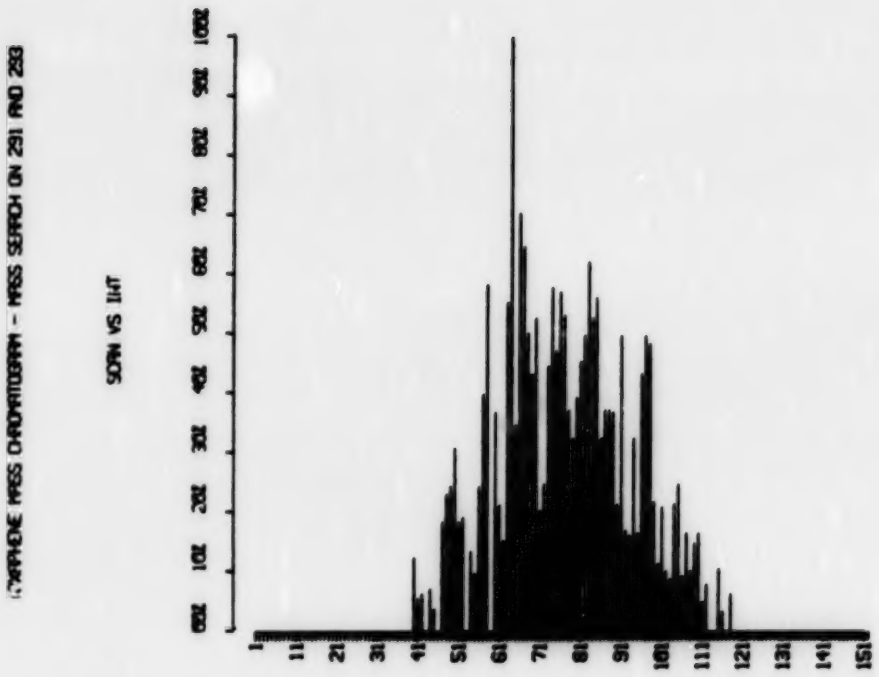

Figure 6. Mass chromatogram from 151 continuous GC-EI-MS scans. Plot of combined ion intensities of m/e 291 + m/e 293.

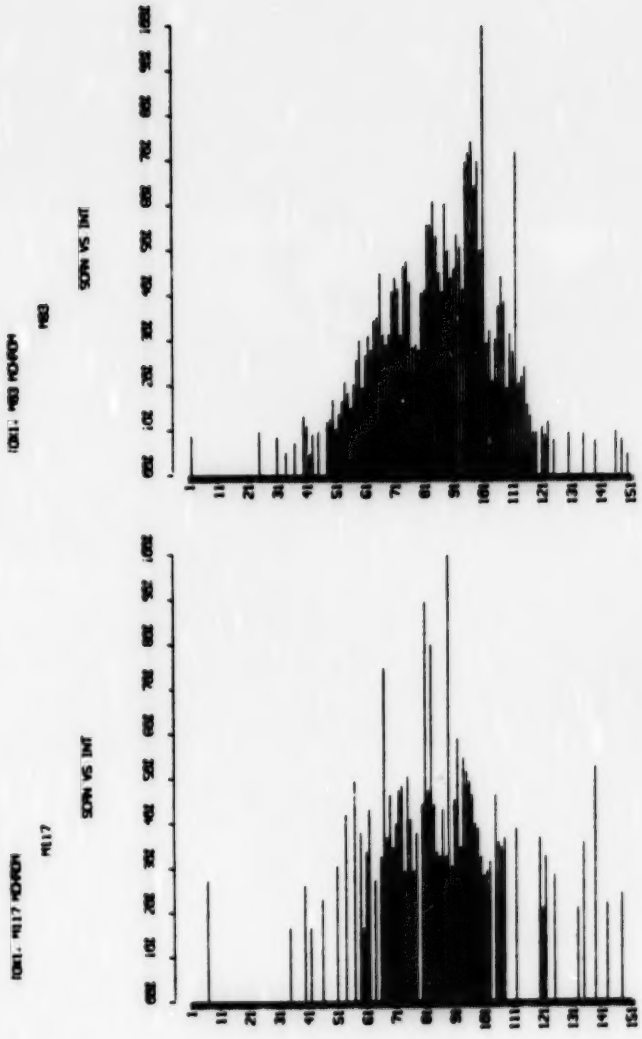

Figure 7. Mass chromatograms from 151 continuous GC-EI-MS scans. Upper plot was of m/e 83, bottom m/e 117.

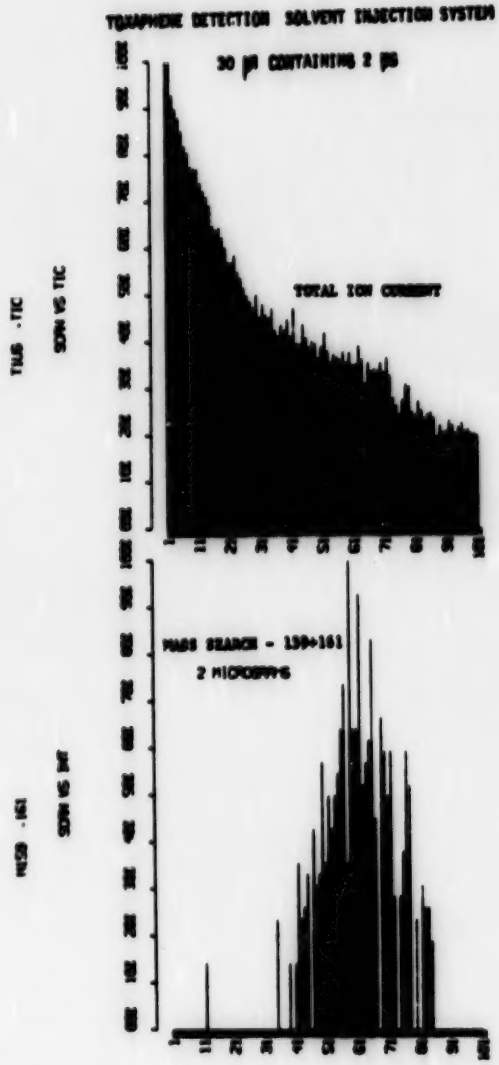

Figure 8. GC-MS large volume, "SOL-VENT" injection of 2 ug of toxaphene in 30 ul. Mass chromatogram from 101 sequential GC-EI-MS scans combining intensity of masses 159 and 161.

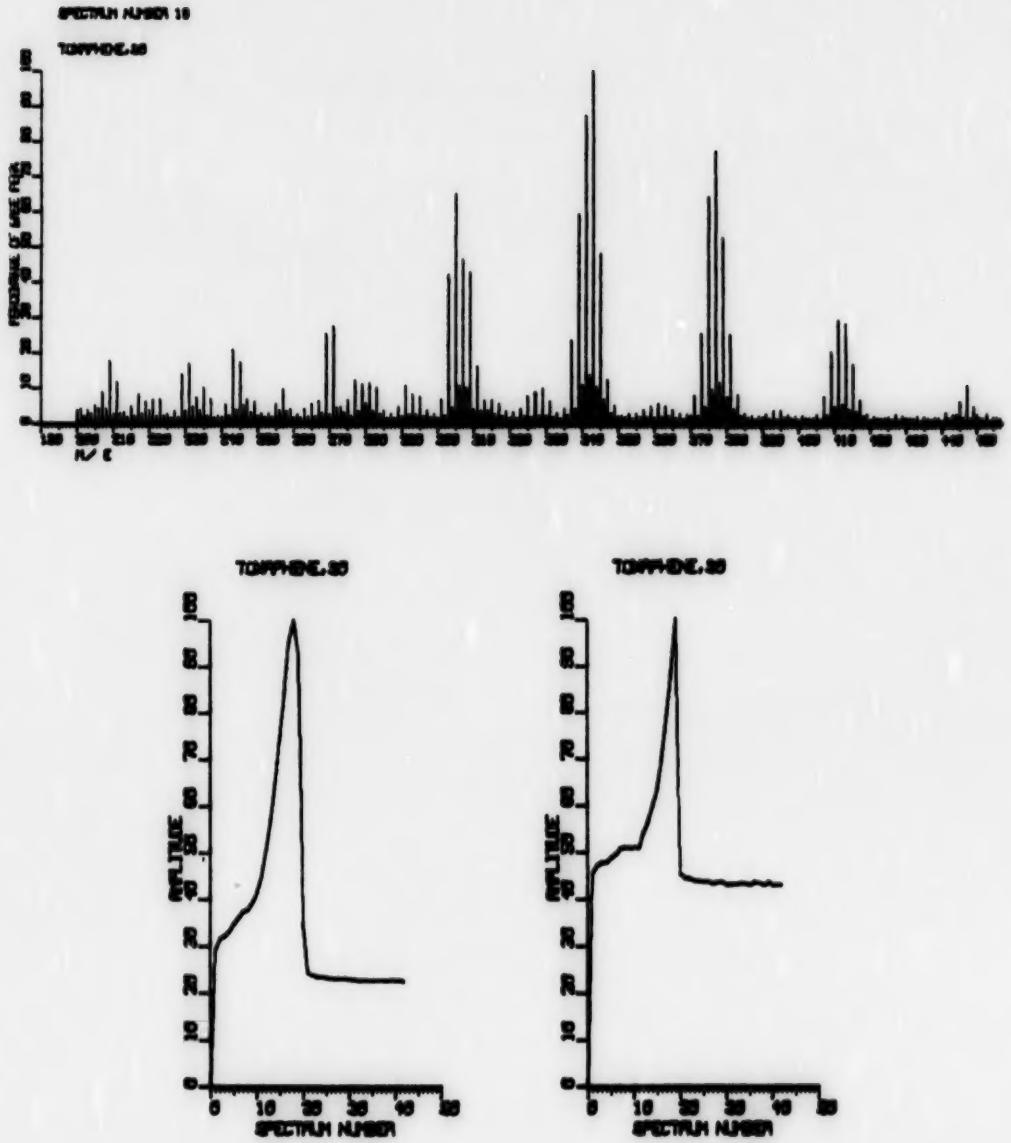

Figure 9. Isobutane-direct probe CI-MS of toxaphene. Cl isotope clusters at 303 represent Cl_5; 341, Cl_6; 374, Cl_7; 409, Cl_8; 445, Cl_9. Toxaphene direct probe CI-TIC plots. Left curve; - TIC for all masses; right curve; - TIC for masses 400-500.

Table 1. RELATIVE CONCENTRATION RATIOS OF CHLORINATED TOXAPHENE CONSTITUENTS DETERMINED FROM THE DIRECT PROBE CI-MS

	Hexa[a]	Hepta	Octa	Nona	Deca
Relative concentration ratio[b]	1.5-2.0	5	4	1-2	1.5-2.0
Ion fragment[c]	305	341	375	409 411	445
Empirical formula[d]	$C_{10}H_{10}Cl_6$	$C_{10}H_{11}Cl_7$	$C_{10}H_{10}Cl_8$	$C_{10}H_9Cl_9$ $C_{10}H_{11}Cl_9$	$C_{10}H_{10}Cl_{10}$

[a]Number of chlorines substituted on camphene ring.

[b]Determined from ratio of intensities of each m/e cluster to sum of isotope cluster intensities for Cl_{6-10}.

[c]Based on ^{35}Cl isotope.

[d]Loss of Cl from parent ion assumed $[M-Cl]^+$

Examination of CI spectra of toxaphene was best approached after a brief review of the molecular weights of several of the possible empirical formulas for increasing chlorination of major constituents (Table 2) and a review of the Cl isotope abundance ratio (Fig. 10). Selected spectra which have been background substracted were plotted in Fig. 10, 11 and 12. The spectrum numbers designated the number of sequential scans and the number of a corresponding spectrum used for substraction of background. These spectra were related to toxaphene GC peaks presented in the GC-CI-MS TIC plot (Fig. 13). The CI-RGC of the TIC of toxaphene (Fig. 13) was similar to that of the EI-MS-TIC (Fig. 14).

The CI-mass spectra of the less complicated portion of the GC curve were characterized by intense $(M-Cl)^+$ ions which reflected the number of chlorine atoms attached to the camphene nucleus. In these cases the empirical formula was readily obtained from the $(M-Cl)^+$ ion. Cl_2 was also lost from the molecule and gave rise to a Cl isotopic cluster at M-70. In many spectra unusual chlorine isotope patterns, when compared to the expected chlorine isotope pattern (Table 2), may only be explained by assuming that GC peaks are mixtures of components which have molecular weights differing by 2 or 4 hydrogens. Thus the mixtures of $C_{10}H_9Cl_9$ and $C_{10}H_{11}Cl_9$ suggested earlier by direct probe CI-mass spectra were corroborated. In addition many other isomers which have one of several empirical formula presented in Table 2 were also indicated.

Two atypical GC-CI-MS toxaphene constituents were noted. GC components examined in scan numbers 76 and 84 had base ion clsuters other than the $M-Cl_2$ fragments (Fig. 15). These mass peaks in scan numbers 76 and 84 were m/e 243 and 245, respectively. M/e 243 corresponded to $C_8H_7Cl_4^+$ and m/e 245 corresponded to $C_8H_9Cl_4^+$. However the ion cluster at m/e 245 was a mixture of $C_8H_7Cl_4^+$ and $C_8H_9Cl_4^+$ (m/e=247) in the ratio of 1:3. Toxaphene RGCs were then obtained for m/e 243 and 245 (Fig. 16). These ion fragments were apparent in all of the CI-MS scans and may reflect the substitution pattern of the toxaphene isomers. While the relative sensitivity of these ions remains to be determined, they are perhaps the most characteristic for toxaphene constituents of the ions we have examined.

Comparisons of toxaphene residues in brook trout with toxaphene standards were made using GC-CI-MS RGCs. The residue in a cleaned up extract from a brook trout exposed to toxaphene for 141 days was examined by GC-CI-MS. Scan numbers of RGCs from the brook trout residue did not directly correspond with those of the toxaphene standard since the computer software required longer scan intervals for lower concentrations of sample. The CI-TIC for the sample extract was plotted in Fig. 17. RGCs for the Cl_7 toxaphene series revealed significant alteration of the sum of toxaphene isomers $C_{10}H_9Cl_7$ (M-Cl=339) + $C_{10}H_{11}Cl_7$ (M-Cl=341) and $C_{10}H_{13}Cl_7$ (M-Cl=343) (Fig. 18). A major constituent in the toxaphene RGC-343 (scan numbers 56 to 57) was greatly reduced in the corresponding RGC-343

scan 101-107 of the brook trout extract. However, comparison of toxaphene and toxaphene residue RGCs for the toxaphene isomer $C_{10}H_9Cl_7$ (M-Cl=339) were more similar (Fig. 19). At least one RGC-339 peak, scan numbers 67-72, from the toxaphene standard was identical to that of the corresponding brook trout RGC-339 peak, scan numbers 121-127.

GC-MS-Computer and in particular GC-CI-MS-Computer permitted characterization of the major constituents of toxaphene. In addition, RGCs or mass chromatograms generated by a GC-MS-Computer system were advantageous tools for the identification of environmental toxaphene residues.

Table 2. EMPIRICAL FORMULA OF TOXAPHENE ISOMERS FROM CI-MS

Cl[a]	Formula	M+H[b]	M-Cl
5	$C_{10}H_9Cl_5$	305	269
	$C_{10}H_{11}Cl_5$	307	271
	$C_{10}H_{13}Cl_5$	309	273
6	$C_{10}H_8Cl_6$	339	303
	$C_{10}H_{10}Cl_6$	341	305
	$C_{10}H_{12}Cl_6$	343	307
7	$C_{10}H_7Cl_7$	373	337
	$C_{10}H_9Cl_7$	375	339
	$C_{10}H_{11}Cl_7$	377	341
8	$C_{10}H_6Cl_8$	407	371
	$C_{10}H_8Cl_8$	409	373
	$C_{10}H_{10}Cl_8$	411	375
9	$C_{10}H_5Cl_9$	441	405
	$C_{10}H_7Cl_9$	443	407
	$C_{10}H_9Cl_9$	445	409
10	$C_{10}H_4Cl_{10}$	475	439
	$C_{10}H_6Cl_{10}$	477	441
	$C_{10}H_8Cl_{10}$	479	443

[a] ^{35}Cl isotope

[b] Mass

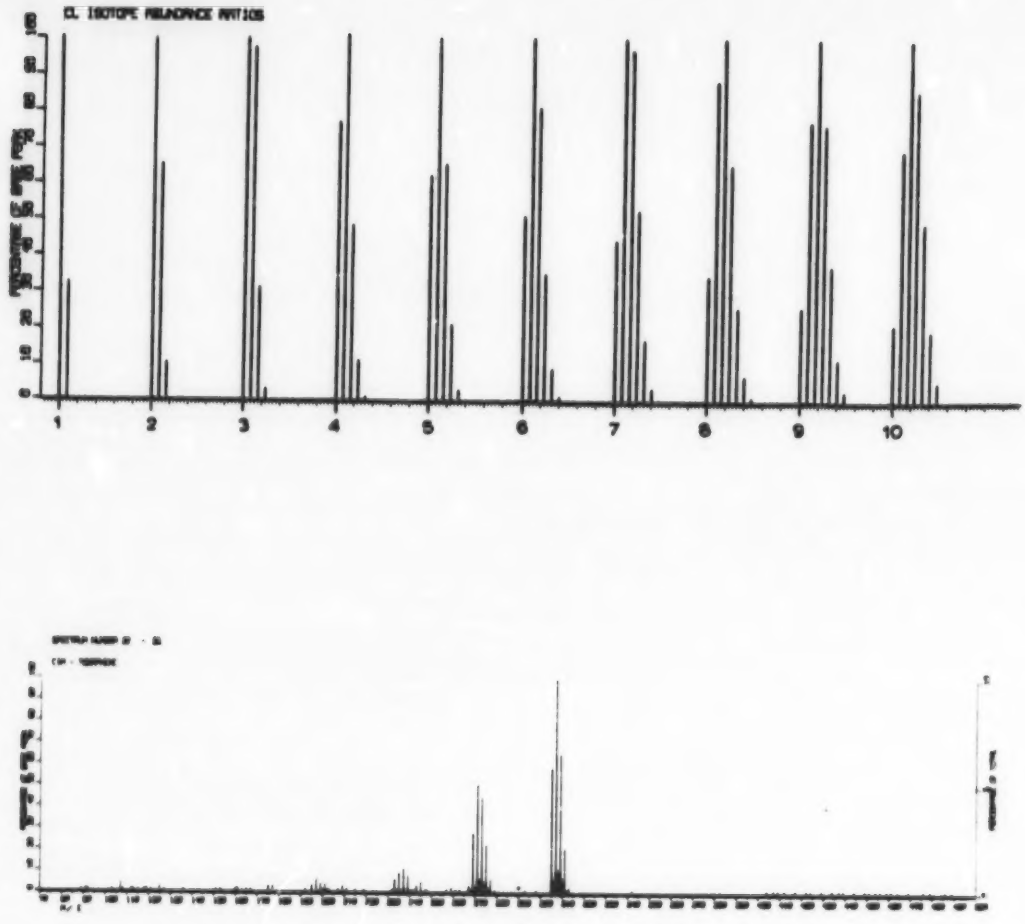

Figure 10. Theoretical Cl isotope patterns for substitution of 1-10 Cl. Calculated from abundance ratio of ^{35}Cl to ^{37}Cl. This information is used when examining chemical ionization spectra of toxaphene to determine if isotope clusters are homogenous. CI-MS spectra of toxaphene GC component. Spectrum, No. 35 subtracted from No. 32. Principal constituents were $C_{10}H_{10}Cl_6$ (M-Cl=305) and $C_{10}H_{11}Cl_5$ (M-Cl=271).

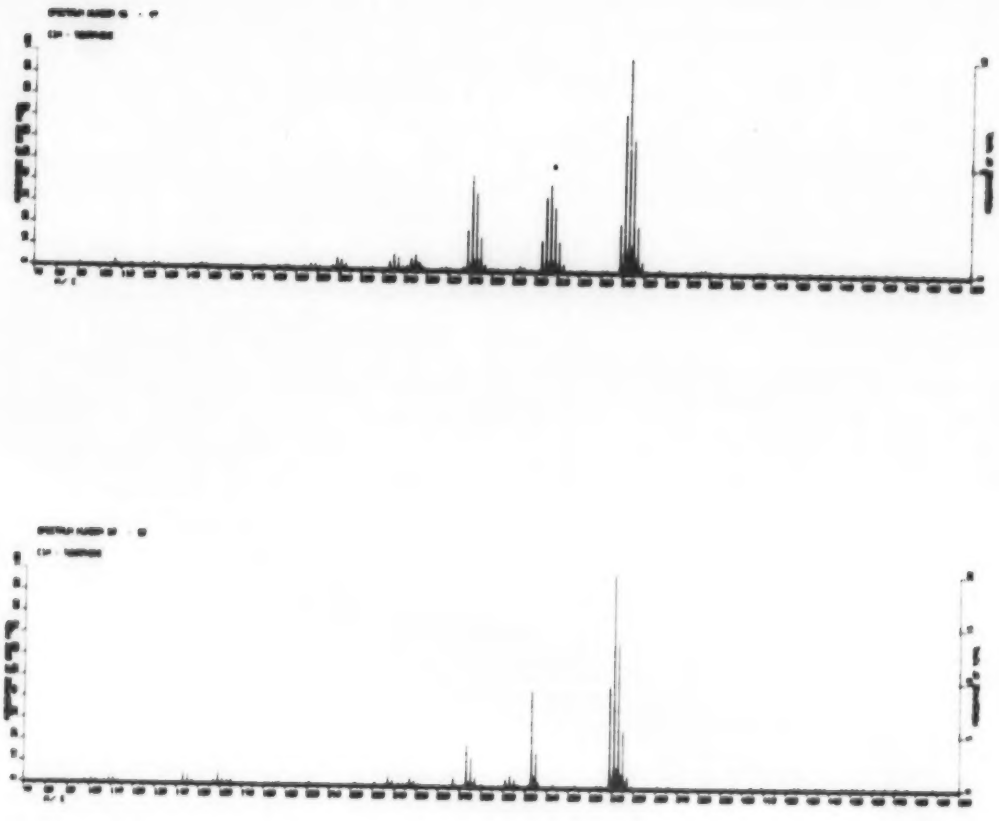

Figure 11. Upper CI-MS of toxaphene constituents. Spectrum, No. 47 subtracted from No. 45. Mixture of $C_{10}H_9Cl_7$ (M-Cl=339) and $C_{10}H_{11}Cl_7$ (M-Cl=341). Note variation of isotope cluster starting at mass 339 for Cl_7. Lower CI-MS also toxaphene constituents. Spectrum No. 52 subtracted from 50. Primarily $C_{10}H_9Cl_7$ (M-Cl=339). Compare isotope cluster at m/e 339 for Cl_7 with corresponding mass isotope cluster in Figure 12.

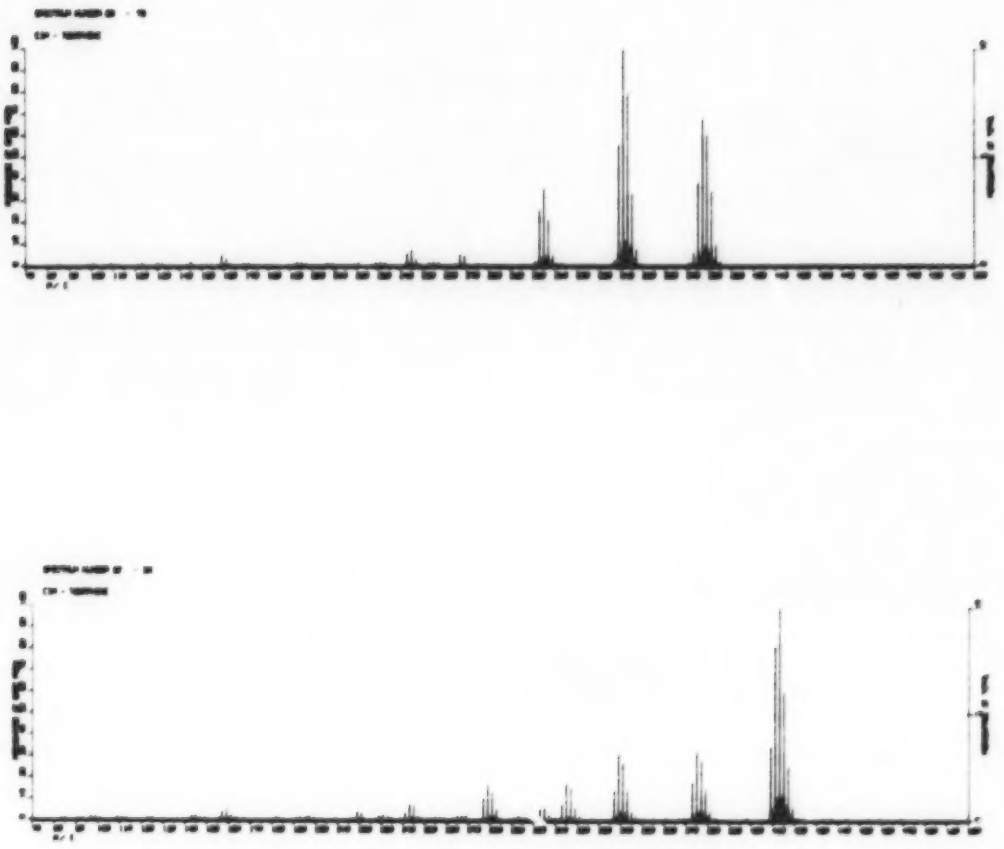

Figure 12. Upper CI-MS of toxaphene constituents. Spectrum No. 73 subtracted from 69. A mixture of $C_{10}H_8Cl_8$ (M-Cl=373) and $C_{10}H_{10}Cl_8$ (M-Cl=375). Relative ratio of mixture 1:20. Lower CI-MS also toxaphene constituents. Spectrum No. 94 minus 92. A mixture of $C_{10}H_9Cl_9$ (M-Cl=409) and $C_{10}H_{11}Cl_9$ (M-Cl=411). Relative ratio of mixture 20:1.

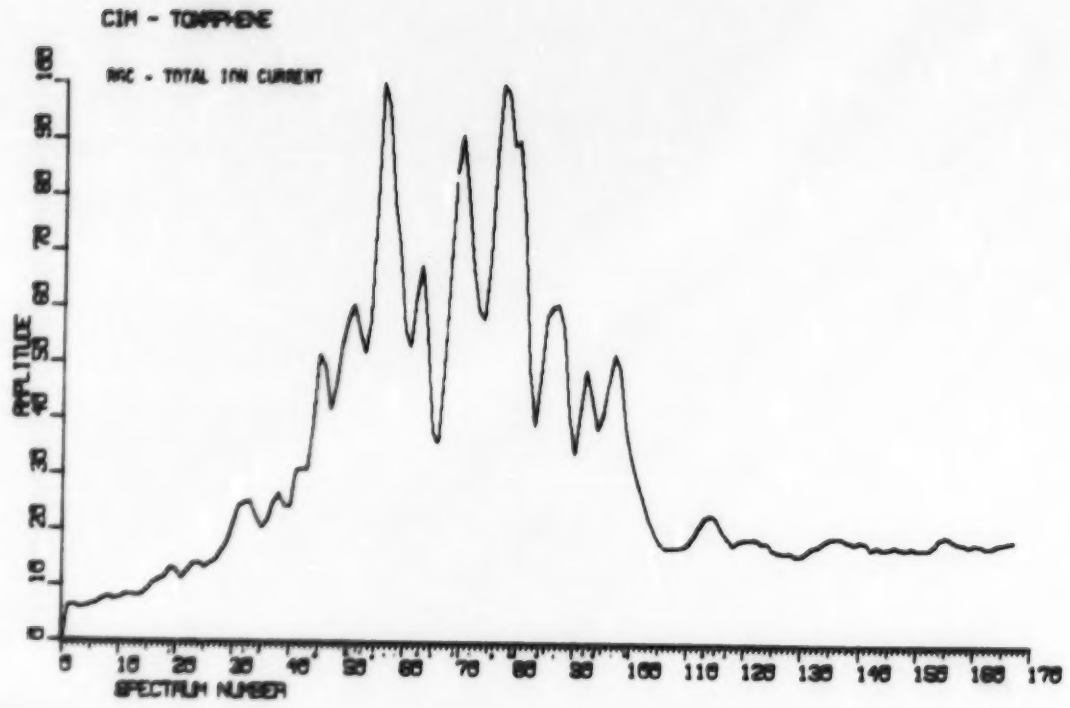

Figure 13. RGC-TIC for GC-CI-MS of toxaphene obtained and plotted using Finnigan-System Industries computer system.

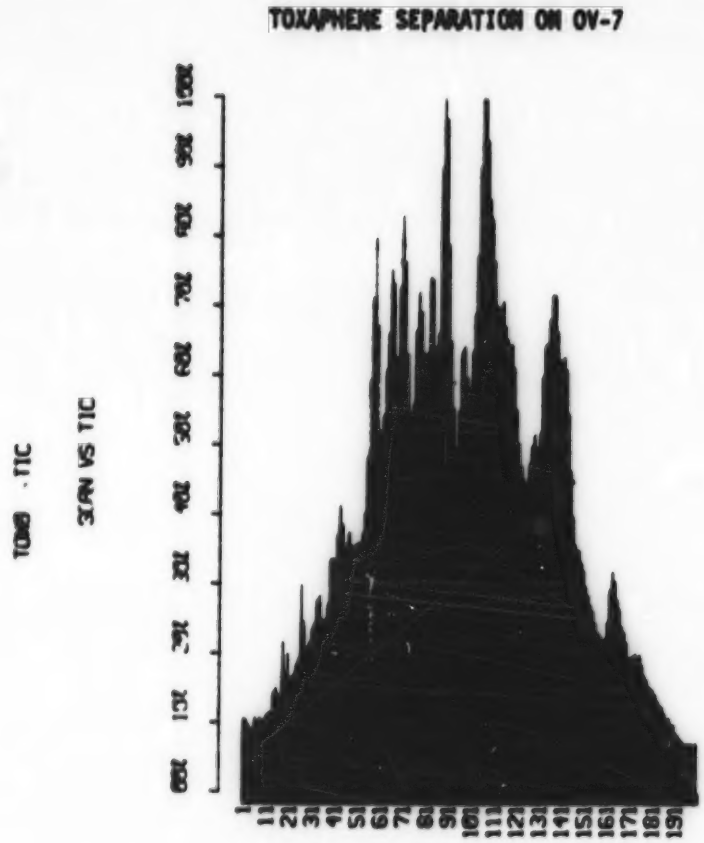

Figure 14. GC-EI-MS-TIC histogram of toxaphene obtained and plotted using Digital Equipment Corporation's MASH computer system.

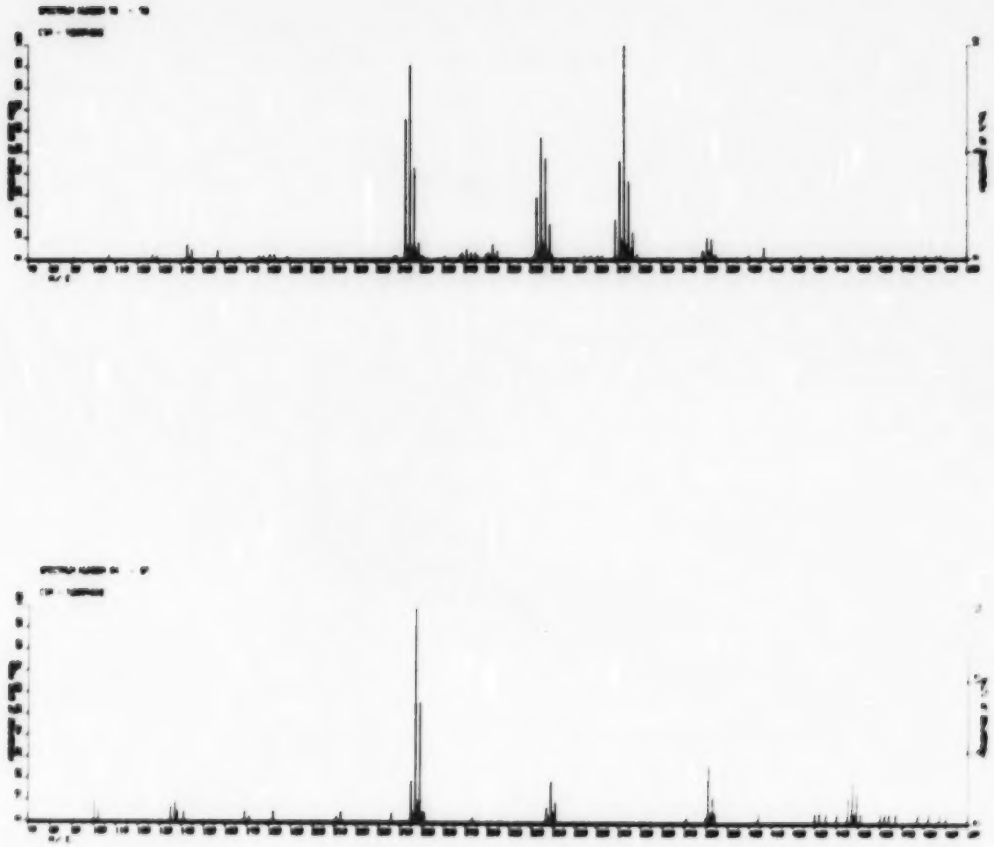

Figure 15. Upper CI-MS of toxaphene constituents ($C_{10}H_9Cl_7$ + $C_{10}H_{11}Cl_7$) having an atypical mass fragment at 243 ($C_8H_7Cl_4^+$). Lower CI-MS also toxaphene constituents having atypical mass fragment $C_8H_9Cl_4^+$ (m/e=245).

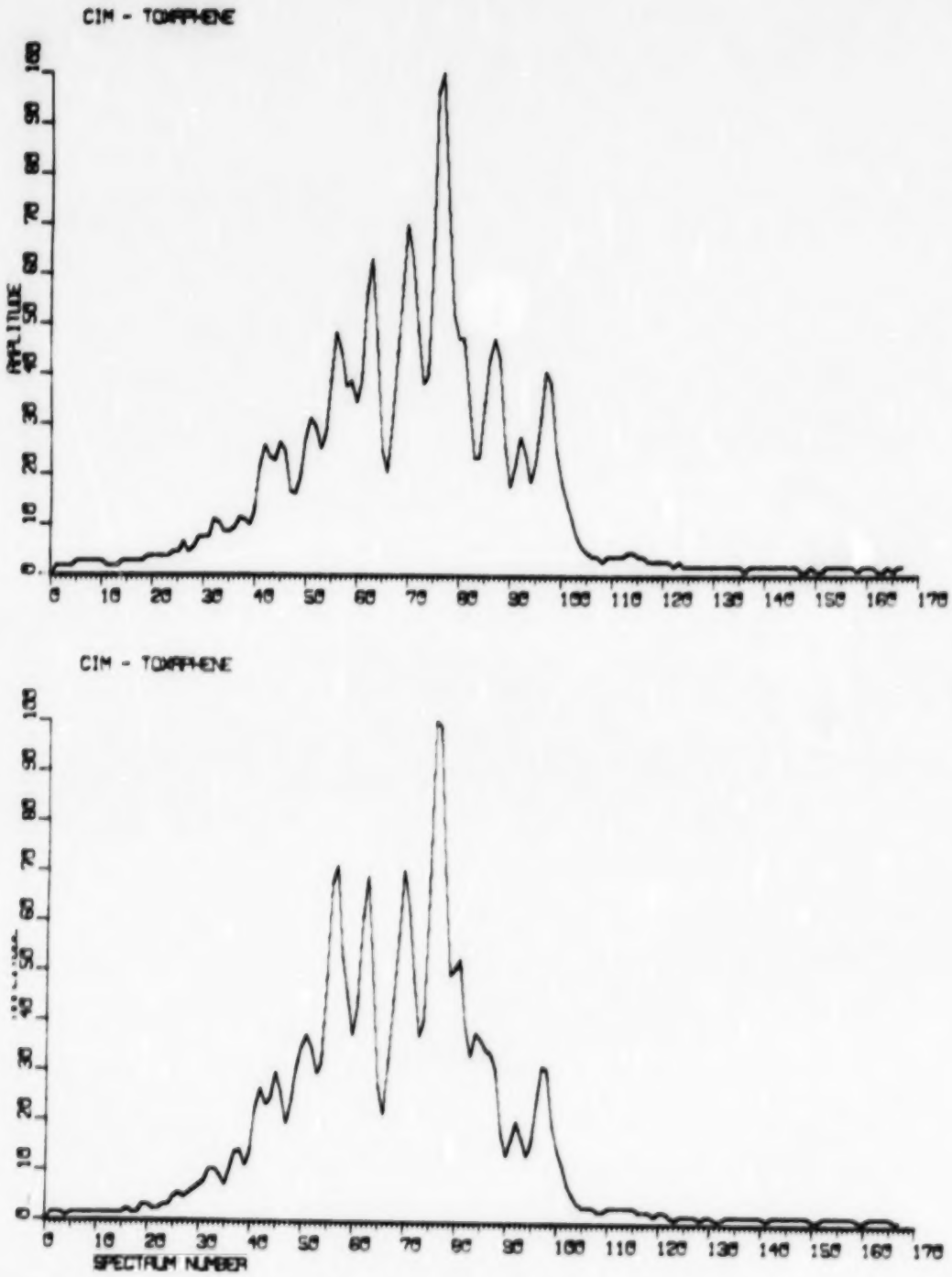

Figure 16. RGC's from CI-MS of toxaphene. Masses scanned were 243 and 245.

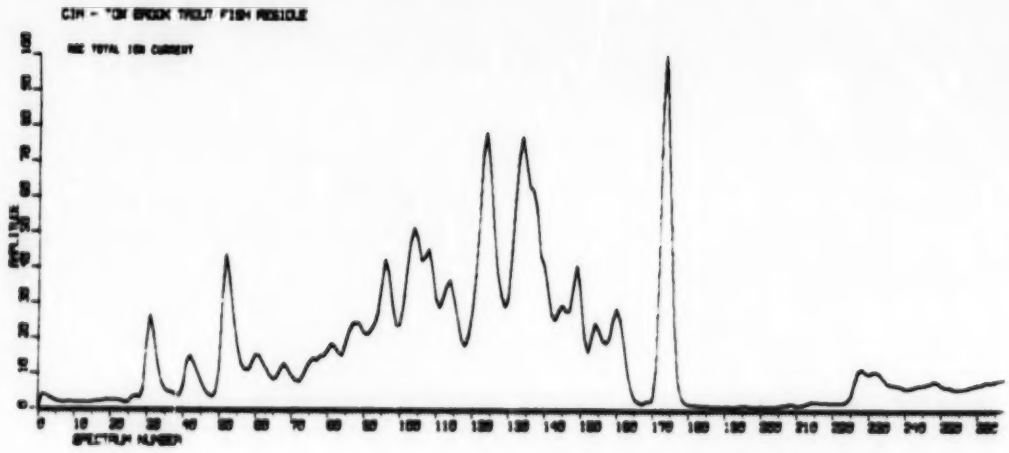

Figure 17. RGC-CI-MS-TIC of an extract from a brook trout exposed to toxaphene for 141 days. Large peak from spectra numbers 165-172 is due to sample contamination by di-2-ethylhexyl phthalate.

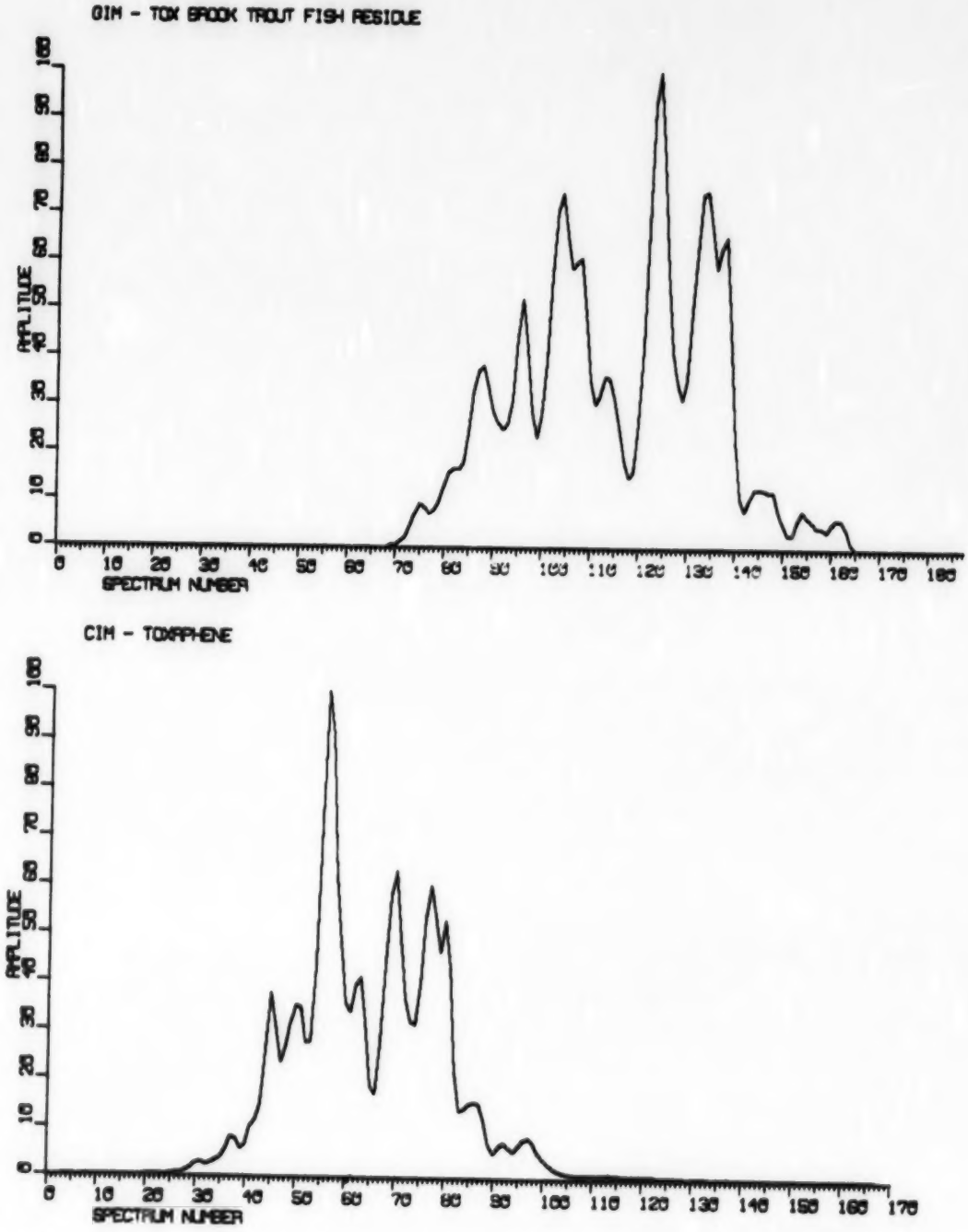

Figure 18. Upper RGC-CI-MS is from an extract of a brook trout exposed to toxaphene for 141 days. Mass scanned was 343. Lower RGC-CI-MS of toxaphene standard, mass scanned was 343.

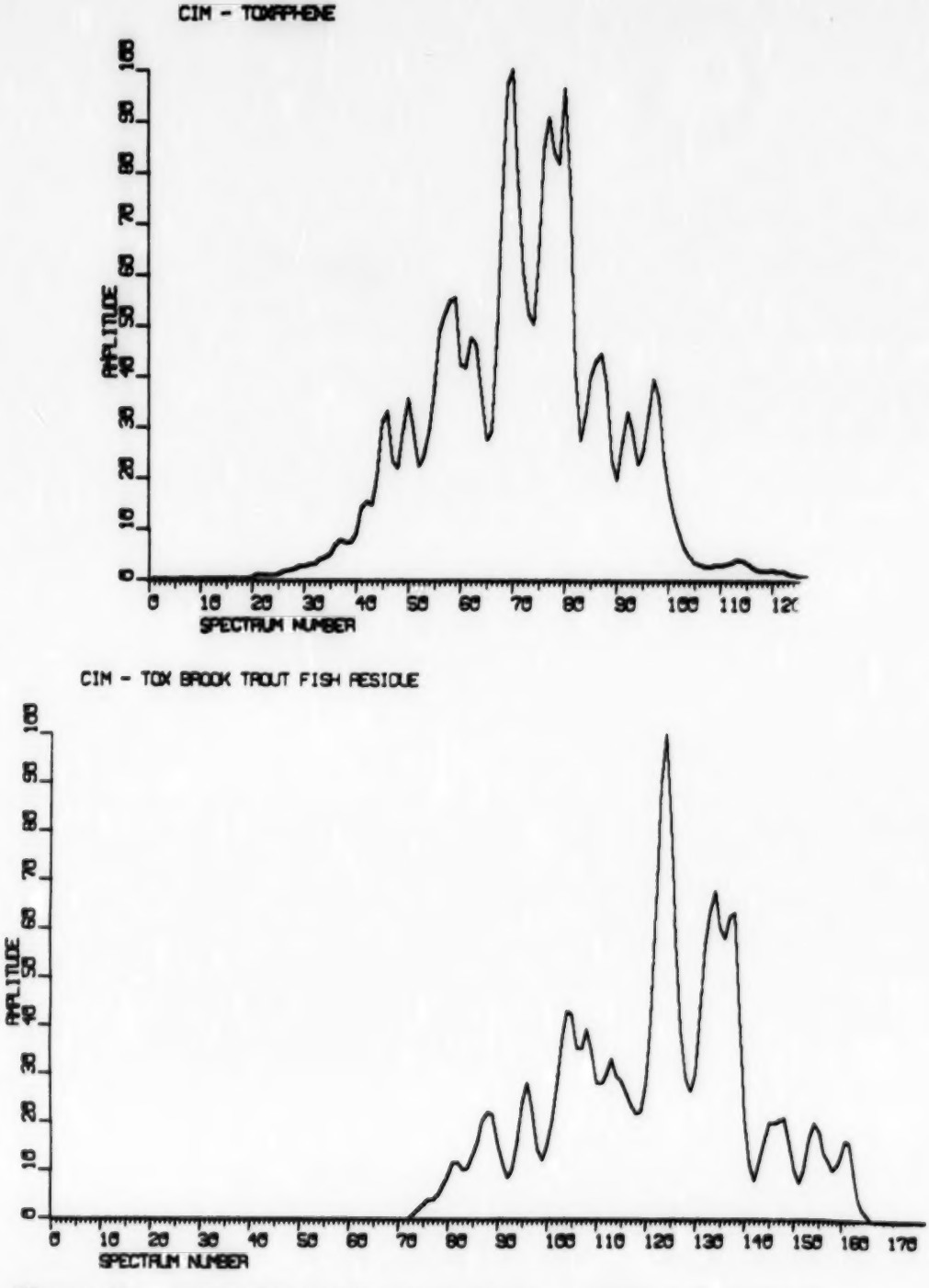

Figure 19. Upper RGC-CI-MS of toxaphene. Mass scanned was 339. Lower RGC-CI-MS of an extract from a brook trout exposed to toxaphene for 141 days. Mass scanned was 339.

SECTION VII

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SECTION VIII

LIST OF PUBLICATIONS

Stalling, D. L. GC-MS Analysis of Toxaphene Residues. 165th American Chemical Society Meeting, Division of Pesticide Chemistry. Dallas, Texas. April 9-13, 1973. Abstract #77.

SECTION IX

GLOSSARY OF ABBREVIATIONS

GC	Gas chromatography
SCOT	Support-coated open tubular
PCB	Polychlorinated biphenyls
EI	Electron impact
CI	Chemical ionization
GC-MS	Combined gas chromatography and mass spectrometry
GC-EI-MS	Combined gas chromatography and electron impact mass spectrometry
GC-CI-MS	Combined gas chromatography and chemical ionization mass spectrometry
TIC	Total ion current
RGC	Computer reconstructed gas chromatogram
M	Mass